



Research Article

ANTIBACTERIAL EFFICACY OF *PLECTRANTHUS AMBOINICUS* EXTRACTS AGAINST STREPTOCOCCUS MUTANS- AN INVITRO STUDY

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ABSTRACT

Background: Dental caries continues to be a major oral health problem affecting people in developing and developed countries. Mechanical plaque control with chemical plaque control as an adjunct is used to prevent biofilm formation. The art and science of utilizing the medicinal properties of the herbs in Ayurveda is well documented. Increased incidences of documented side effects of the allopathic drugs with ever increasing cost of the same has paved way for a renewed interest in the concept of Ayurveda. *Plectranthus amboinicus*, a medical plants known to possess antimicrobial, antiepileptic and antioxidant properties. It has proven efficacy against a host of micro-organisms and may also be effective against *Streptococcus mutans*. **Aim:** The present study aims to find out the antibacterial efficacy of *Plectranthus amboinicus* extract against *Streptococcus mutans*. **Materials and methods:** Aqueous and ethanolic leaf extracts of Black *Plectranthus amboinicus* were prepared. The antibacterial efficacy was tested against *Streptococcus mutans* through Disc Diffusion Test and Minimum Inhibitory Concentration (MIC) Test. **Results:** *S. mutans* was found to be sensitive to ethanolic and aqueous extracts of *Plectranthus amboinicus* at two dilutions with MIC 50 µg/ml. **Conclusion:** The ethanolic and aqueous extracts of *Plectranthus Amboinicus* exhibited antibacterial effect against *Streptococcus Mutans* in vitro.

KEYWORDS: Antibacterial Efficacy, *Plectranthus Amboinicus* Extracts, *Streptococcus Mutans*.

INTRODUCTION

The art and science of utilizing the medicinal properties of the herbs in Ayurveda is well documented. Increased incidences of documented side effects of the allopathic drugs with ever increasing cost of the same has paved way for a renewed interest in the concept of Ayurveda. The medical properties of the plants are based on the antioxidant, antipyretic, antimicrobial and/or analgesic properties of certain phytochemicals in them. Of the several medicinal plants used in the science of Ayurveda, *Plectranthus amboinicus*, (synonym: *Coleus amboinicus*, *Coleus aromaticus*) commonly known as Country borage, Indian borage or Patharchat, *Karpooravalli*, *Sugandhavalakam* in Sanskrit⁽¹⁾, is a dicotyledonous plant belonging to the family Lamiaceae, is known to possess antimicrobial, antiepileptic and antioxidant properties.⁽²⁻⁵⁾

Dental caries continues to be a major oral health problem affecting people in developing and developed countries. The occurrence of dental caries is directly linked to the ability of microorganisms to colonize the tooth surface and form biofilm. *Streptococcus mutans*, *Lactobacilli* and *Candida albicans* are the predominant microorganisms found in dental plaque associated with a caries lesion.⁽⁶⁾ Mechanical plaque control with chemical plaque control as an adjunct is used to prevent biofilm

formation. Among the evaluated agents for chemical control, Chlorhexidine (CHX) is the gold standard.⁽⁷⁾ But there are few drawbacks in using Chlorhexidine like alteration in the taste perception, staining of the teeth and desquamation of the oral mucosa.⁽⁷⁾ Therefore, mild but effective agents are required for adjuvant oral biofilm management without negative effects on the ecology of the oral cavity. *Plectranthus amboinicus* having proven efficacy against a host of micro-organisms may also be effective against *Streptococcus mutans*. Literature search revealed no studies conducted to assess the antibacterial activity of *Plectranthus amboinicus* against *Streptococcus mutans*. Hence, an attempt was made to find out the antibacterial efficacy of *Plectranthus amboinicus* extract against *Streptococcus mutans* with the objective to assess the zone of inhibition and minimum inhibitory concentration of *Plectranthus amboinicus* extract against *Streptococcus mutans*.

MATERIALS AND METHODS

Study design

Experimental design. In- vitro study. Laboratory setting, Bapuji Dental College and Hospital Dvanger.

Method

The methodology for preparation of the extract, stock solution and the microbiological procedures were adopted from a study done by Preeja G. Pillai et al. ⁽⁸⁾

Preparation of Extract



The leaf extract of *Plectranthus amboinicus* was prepared by cold maceration method. Black *Plectranthus amboinicus* plant was identified based on the taxonomic features by a botanist. The leaves were collected from the identified plants, cleaned with distilled water and shade dried. The dried leaves were finely powdered with an electric grinder. 100 grams of the leaf powder was weighed and macerated in 600 ml of distilled water in a sterile glass container for aqueous extract and 100 grams of the leaf powder was weighed and macerated in 600 ml of ethanol in a sterile glass container for ethanolic extract. Cold maceration was performed for two days by shaking at regular intervals. It was subjected to filtration using Whatman filter paper to obtain a clear filtrate. This was kept on a water bath set at 60° C to obtain crude extract of *Plectranthus amboinicus* leaves.

Cultivation of microorganisms

Streptococcus mutans was grown on 85mm blood agar plates. A suspension was prepared by transferring the colonies from blood agar plates using a sterile platinum loop to 10ml of brain heart infusion agar. Later it was inoculated for one day at 37°C. Several dilutions were prepared to obtain a standardized number of cells per one micro litre of the culture medium using the Serial dilution technique.

Assessment of antibacterial activity

Pure strains of *S. mutans* (ATCC 25175) were used in the study. The antibacterial activity of extracts

was evaluated by using the Disc diffusion test and Minimum inhibitory concentration test.

1. Disc diffusion test (Disc diffusion assay)

A modified agar diffusion method was used to determine antimicrobial activity. Nutrient agar was inoculated with a microbial cell suspension (200 µl in 20 ml of medium) and poured into sterile petridishes. Sterile filter paper disc 6mm in diameter was impregnated with 20 µl of each extract concentration. Concentrations were prepared using the same solvent as employed to dissolve the *Plectranthus amboinicus* leaves (ethanol, Ethyle acetate and distilled water), later sterilized via pasteurization and membrane filtration (regarding the aqueous extract), and placed on the inoculated agar surface. After pre-incubation for 2 hours in a refrigerator the plates were incubated overnight at 37°C for 18 days. The zone of inhibition of *streptococcus mutans* was assessed in millimetres.



2. Minimum Inhibitory Concentration Test (Micro-Dilution Assay)

The minimum inhibitory concentration (MIC) values were determined in terms of microliter (µl) with the agar dilution method in 96 multi- well micro titre plates, as previously described. The dissolved extracts were first diluted to the highest concentration to be tested (12.5 mg/ml), 50 µl of nutrient broth was distributed from the 2nd to the 12th well, a volume of 100 µl from each of the ethanol, ethyl acetate and aqueous extracts were initially prepared and pipetted into the 1st test wells of each micro titre line, and then 50 µl of dilution was transferred from the 2nd to the 12th well. The final concentration of the extracts adopted to evaluate antibacterial activity was included from 50 mg/ml to 0.12 mg/ml (from 1st dilution to the 10th dilution). Plates were wrapped loosely with cling film to ensure that bacteria did not become dehydrated and then they were placed in an incubator at 37°C for 18-24 hours. Colour change was then assessed visually. Any colour change from purple to pink or colourless was recorded as positive. The lowest concentration at which colour change occurred was taken as MIC value.

RESULTS

Table 1: Zone of inhibition of leaf extract of *Plectranthus amboinicus* against *Streptococcus mutans*

Form of leaf extract	Volume of the test agent in each well				
	75µl	50 µl	25 µl	10 µl	5 µl
Ethanol	R	R	R	R	R
Aqueous	R	R	R	R	R

S-Sensitive; R-Resistant

Table 2: Minimum inhibitory concentration of *Plectranthus amboinicus* against *Streptococcus mutans*

Form of leaf extract	Concentrations to which the test agent was diluted in µg/ml									
	100	50	25	12.5	6.25	3.125	1.6	0.8	0.4	0.2
Ethanol	S	S	R	R	R	R	R	R	R	R
Aqueous	S	S	R	R	R	R	R	R	R	R

S-Sensitive; R-Resistant

RESULTS

Zone of inhibition

Both ethanolic and aqueous extracts of *Plectranthus Amboinicus* did not show any inhibition zones against *Streptococcus Mutans* when assessed using different volumes of the test agents. (Table 1)

Minimum Inhibitory Concentration

S. mutans was found to be sensitive to ethanolic and aqueous extracts of *Plectranthus amboinicus* till two dilutions and hence the MIC was 50 µg/ml. (Table 2)

DISCUSSION

Dental caries is still a public health problem both in developed as well as developing countries owing to its universal distribution and the impact it has on the individual and the society. Preventing dental caries seems to be the most appealing modality to reduce the burden of the disease. One of the targets for preventing dental caries is to reduce the load of *Streptococcus mutans* count.

In the recent years there is a surge in interest towards indigenous medicine- Ayurveda. One among the various Ayurvedic products is *Plectranthus amboinicus* (Lour). It is a tender, fleshy perennial herb. The extract of this plant was prepared and its invitro antibacterial activity against *Streptococcus mutans* was assessed.

To our best knowledge present study is first of its kind hence valid comparisons could not be established. The extract of the leaves was prepared based on the study conducted by Preeja et al.⁽⁸⁾ Cold maceration technique was employed to prepare the extracts to ensure that the heat sensitive active agents present in the *Plectranthus amboinicus* leaves are not degraded during extraction process.

It was interesting to note that *S. mutans* were sensitive to the *Plectranthus amboinicus* leaves extract at 50 µg/ml, however, no zone of inhibition was observed in agar diffusion method. Agar diffusion

method is a standard method to assess the invitro antimicrobial property of the drug. The zone of inhibition of micro-organisms depends on its diffusion capacity through the solid agar medium. One of the possibilities is that, though the extracts of *Plectranthus amboinicus* leaves exhibited antibacterial property against *S. mutans*, it might not possess the required physical property to diffuse through solid agar medium.

In the present study both aqueous and ethanolic extracts of *Plectranthus amboinicus* had antibacterial property against *Streptococcus mutans* species at 50 µg/ml concentration. The selection of the *Plectranthus amboinicus* leaves in the present study was based on number of factors. The leaves have been traditionally used for the treatment of chronic coughs, cold, bronchitis, asthma, nasal congestion as well as diarrhoea. It can help in the treatment of cancer as it has anti-tumour and cytotoxic activities. Hence their potential can be harnessed against *Streptococcus mutans*. The leaves are readily available, cheap and acceptable by the people and of great relevance to Indian scenario.

CONCLUSIONS

In the present study, disc diffusion test demonstrated no antibacterial effect of *Plectranthus amboinicus* leaves extract against *S. mutans*. However, in micro dilution assay test, both the aqueous and ethanolic extract of *Plectranthus amboinicus* leaves exhibited antibacterial effect against *S. mutans* at two dilutions (50 µg/ml concentration). Further invitro and invivo studies have to be conducted to test the anticariogenic potential of this herb. Hence, if proven effective against *S. mutans*, it can be a very useful indigenous anticariogenic agent.

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